and wherein the biologically active ribonucleic acids or peptides or cellular ligands to the biologically active ribonucleic acids or peptides are identified by

e) isolating and sequencing the vector DNA in the cells having altered phenotypic trait, and deducing, from the sequenced vector DNA, the sequences of the ribonucleic acids or peptides being expression products of the synthetic totally random DNA from cells exhibiting alteration of the preselected phenotypic trait; and/or using directly, for isolation and identification of a ligand molecule to said ribonucleic acids or peptides, the ribonucleic acids or peptides containing expression products of the synthetic totally random DNA from cells exhibiting alteration of the preselected phenotypic trait.

72. The method according to claim 71, in which the peptide is a peptide sequence inserted into or fused to a protein amino acid sequence.

73. The method according to claim 71, in which the synthetic totally random DNA sequences are produced by random codon synthesis, where defined DNA codons are synthesized in a random order.

74. The method according to claim 71, in which the synthetic totally random DNA sequences are produced by conventional random oligonucleotide synthesis.

75. The method according to claim 71, in which the synthetic totally random DNA sequences are introduced into the expression vector in step (a) by site directed PCR-mediated mutagenesis thereby ensuring the complexity of the totally random DNA sequences.

76. The method according to claim 75, in which optimal combining efficiencies of two PCR products is achieved by trimming 3' ends of PCR products with a 3'-5' exonuclease.

77. The method according to claim 71, in which the step of producing the appropriate vectors comprises ligating a DNA fragment into a vector in an optimized manner by performing temperature cycling ligation in step (a), thereby maintaining a high diversity of the totally random DNA sequences for transfection into packaging cells.

78. The method according to claim 71, in which the synthetic totally random DNA sequences are introduced into the number of identical eukaryotic cells in such a way that only one DNA sequence is introduced in each cell, one cell expressing one ribonucleic acid and possibly one peptide, thus enabling a particular sequence to be isolated and analyzed.

79. The method according to claim 71, in which the synthetic totally random DNA sequences are introduced into the eukaryotic cells by use of an appropriate viral vector selected from the group consisting of a retrovirus vector and a vaccinia virus vector.

80. The method according to claim 79, in which the vector used is a retroviral vector.

81. The method according to claim 80, in which the retroviral vector has heterologous ends to facilitate PCR-based generation of the synthetic totally random DNA sequences.

82. The method according to claim 81, in which the heterologous ends contain two different promoters.

83. The method according to claim 80, in which the retroviral vector contains a CMV promoter replacing the viral promoter in the 5'-LTR.

84. The method according to claim 79, in which the synthetic totally random DNA sequences are produced as linear PCR products where are directly introduced into virus packaging cells by non-viral transfection methods.

85. The method according to claim 79, in which viral vector DNA introduced into the cells is amplified directly by polymerase chain reaction followed by transfection of further cells with amplified vector DNA with the purpose of eliminating false positives and/or enabling the "one cell - one ribonucleic acid or peptide" concept.

86. The method according to claim 79, in which retroviral packaging cell line viral titer is increased by transient transfection with a functional tRNA gene corresponding to a primer binding site (PBS) in the vector.

87. The method according to claim 79, wherein the appropriate viral vector is produced by a packaging cell line transfected with a vector encoding one single transcript which translates into gag-pol, a selectable marker, and env.

88. The method according to claim 79, wherein the appropriate viral vector is produced by a semi-packaging cell line transfected with a suitable minivirus/vector thereby enabling vector expression after transduction of cells rather than after transfection of cells.

89. The method according to claim 71, in which the peptides being expression products from cells exhibiting alteration of the preselected cellular function also contain a purification tag which enables direct isolation of the peptide as well as of the molecule with which the peptide interacts.

90. The method according to claim 71, in which appropriate signal peptides, other leader molecules or recognition sequences are also encoded by the vectors in the form of fusion partners to peptides encoded by the synthetic totally random DNA.

91. The method according to claim 71, in which the synthetic totally random DNA sequences are inserted into, or linked to a DNA sequence encoding a protein expressed simultaneously from the vectors.

92. The method according to claim 91, in which the protein is selected from the group consisting of secreted proteins, intracellular proteins, and membrane proteins.

93. The method according to claim 91, in which the protein is a heavy and/or light chain of an antibody molecule, or a part thereof.

94. The method according to claim 71, wherein screening in step (c) identifies presence of T-cell epitopes bound to MHC molecules on the surface of the transduced cells.

95. The method according to claim 71, wherein the alteration of the preselected cellular function is up-regulation or down-regulation of expression of a cell surface protein.

96. The method according to claim 71, wherein the synthetic totally random DNA sequences each encode 6-10 random amino acids.

97. The method according to claim 96, wherein the synthetic totally random DNA sequences each encode 8-9 random amino acids.

98. The method according to claim 72, wherein the protein amino acid sequence is selected from the group consisting of an F(ab) fragment amino acid sequence and an antibody molecule amino acid sequence.

99. The method according to claim 71, wherein the synthetic totally random DNA sequences are separated by codons encoding glycosylation sites.

100. The method according to claim 79, wherein the viral vector is a vaccinia virus vector.

101. In a drug development method wherein a lead compound serves as starting point for design and synthesis of candidate drugs, the improvement comprising that a ribonucleic acid or peptide which has been identified according to claim 71 is the lead compound.

102. The method according to claim 71, wherein the ribonucleic acids or peptides being expression products from cells exhibiting alteration of the preselected phenotypic trait are used directly for isolation of a cellular target protein of the identical cells to said ribonucleic acids or peptides.

103. The method according to claim 71, wherein the eukaryotic cells are mammalian.

104. The method according to claim 71, wherein the identical eukaryotic cells are cells of a cell clone or a cell line.

105. The method according to claim 71, wherein the synthetic totally random DNA sequences do not include stop codons.

106. The method according to claim 71, wherein the synthetic totally random DNA sequences encode random amino acid sequences with an even distribution of amino acids.

107. The method according to claim 71, wherein the synthetic totally random DNA sequences are separated by codons which encode specific post-translational modifications of all expressed peptides or which encode anchor residues.

108. The method according to claim 71, wherein the synthetic totally random DNA sequences are coupled to coding sequences of purification tags in order to facilitate the purification and identification of expressed peptides.

109. The method according to claim 71, wherein the synthetic totally random DNA sequences are coupled to or inserted into the coding sequence of a protein.

110. The method according to claim 72, in which appropriate signal peptides, other leader molecules or recognition sequences are also encoded by the vectors in the form of fusion partners to the protein amino acid sequence, thereby enabling translocation of the protein amino acid sequence.

111. A method for identification of biologically active ribonucleic acids or peptides or cellular ligands to the biologically active ribonucleic acids or peptides, which comprises the steps of

a) producing a pool of appropriate vectors each containing a DNA sequence to be examined,

b) efficiently transducing said vectors into a number of identical eukaryotic cells in such a way that each cell expresses either a single ribonucleic acid and possibly peptide encoded by the DNA sequence to be examined or a limited number of different ribonucleic acids and peptides encoded by DNA sequences to be examined,

c) screening said transduced cells to see whether some of them exhibits up-regulation or down-regulation of a preselected cellular function, and

d) selecting and cloning cells which have up-regulated or down-regulated the preselected cellular function,

wherein each vector in the pool of appropriate vectors in step (a) contains a synthetic totally random DNA sequence,

and wherein the biologically active ribonucleic acids or peptides or cellular ligands to the biologically active ribonucleic acids or peptides are identified by

e) isolating and sequencing the vector DNA in the selected and cloned cells, and deducing, from the sequenced vector DNA, the sequences of the ribonucleic acids or peptides being expression products of the synthetic totally random DNA from cells exhibiting up-regulation or down-regulation of the preselected cellular function; and/or using directly, for isolation and identification of a ligand molecule to said ribonucleic acids or peptides, the ribonucleic acids or peptides containing expression products of the synthetic totally random DNA from cells exhibiting up-regulation or down-regulation of the preselected

cellular function for isolation and identification of a ligand molecule to said ribonucleic acids or peptides.

112. The method according to claim 111, wherein the synthetic totally random DNA sequences do not include stop codons.

113. The method according to claim 111, wherein the synthetic totally random DNA sequences encode random amino acid sequences with an even distribution of amino acids.

114. The method according to claim 111, wherein the synthetic totally random DNA sequences are separated by codons which encode specific post-translational modifications of all expressed peptides or which encode anchor residues.

115. The method according to claim 111, wherein the synthetic totally random DNA sequences are coupled to coding sequences of purification tags in order to facilitate the purification and identification of expressed peptides.

116. The method according to claim 111, wherein the synthetic totally random DNA sequences are coupled to or inserted into the coding sequence of a protein.

117. The method for isolation of a cellular target protein which has influence on a preselected cellular function in a eukaryotic cell, which comprises the steps of

a) producing a pool of appropriate vectors each containing a DNA sequence to be examined, wherein each vector in the pool contains a synthetic totally random DNA sequence,

b) efficiently transducing said vectors into a number of identical eukaryotic cells in such a way that each cell expresses either a single ribonucleic acid and possibly peptide encoded by the DNA sequence to be examined or a limited number of different ribonucleic acids or possibly peptides encoded by DNA sequences to be examined,

c) screening said transduced cells to see whether some of them have altered the preselected cellular function after step (b),

d) selecting and cloning cells which have altered the preselected cellular function,

e) isolating the cellular target protein by using, as an affinity ligand, a ribonucleic acid or peptide comprising an expression product of the synthetic totally random DNA isolated from a cell exhibiting alteration of the preselected cellular function.--

REMARKS

New claims 71-117 are presented for consideration.